Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (1):233-236
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Chemical analysis and antibacterial activity of the ethanolic extract of *Stenochlaena palustris*

[1]Erwin*, [1]Devi Anggeraini and [2]Suryani

[1]Department of Chemistry, Faculty of Mathematic and Natural Sciences, Mulawarman University, Jalan Barong Tongkok No. 4, Samarinda, Indonesia
[2]Department of Chemical Processing Engineering, Politeknik ATI Makassar, Makassar, Indonesia

---

ABSTRACT

Stenochlaena palustris (local name: Kalakai) is an edible ferns, that found in the tropical and subtropical forest. It known by the people as a highly nutritious vegetable. This study was conducted to determine the chemical compounds from ethanol extract of Kalakai using gas chromatography-mass spectrometry (GC-MS) technique and antibacterial activity against Staphylococcus aureus dan Eschrichia coli. Interpretation of GC-MS spectrum showed four chemical compounds. Stigmasterol and its derivatives are the major compounds (total 97.47%) and ethyl linoleate is a minor compound (2.57%). Stigmasterol has various interesting bioactivity based on a literature review. While the value of minimum inhibition concentration (MIC) of ethanol extract of Kalakai is 10% (w/v) against both Staphylococcus aureus and Eschrichia coli.

Keywords: *Kalakai*, GC-MS spectrometry, bioactivity, and antibacterial activity

---

INTRODUCTION

Stenochlaena is a small genus of six (or possibly seven) species confined to the old-world tropics and sub-tropics [1]. *Stenochlaena palustris* (local name : *Kalakai*), one of the plants of this genus, most widely distributed, best known, abundant, and variable species, occurs throughout Malesia, extending northwest to India, north to other south-east Asian countries (including the Indonesian Islands), south to northern Australia and east to the Bismarck Archipelago of Papua New Guinea and the south-west Pacific region [1,2].

Kalakai grow vegetatively with a fairly high ability. There are differences in growth rate between the dry seasons with the rainy season. In the dry season *kalakai* is slower growth rate than the rainy season. This is due to differences in the ability to produce biomass and the limited amount of water that can be utilized [3]. This plant has a relatively short harvest period (4-6 days) means that within this time period can be re-harvest and grow well in areas that have high humidity such as peat lands [4]. Fiddleheads of ferns are also used as a vegetable in the South Pacific region and in India [5]. In East Kalimantan easily leaves of this plant were sold in traditional markets, and it was cooked as a vegetable. *Kalakai* is traditionally by the people in Central Kalimantan recognized to stimulate the production of breast milk in post-delivery mothers [6]. Extracts of *kalakai* leaves is potent antioxidant [7], decrease the CEC in the marmot plasma [8] and higher antimicrobial properties [9]. It is also potential as a safe natural alternative preservative-based food product for future generations [10], an antioxidant activity by chelating effect on

ferrous ions, hydroxyl radical scavenging, and hydrogen peroxide scavenging [11] and potential as source of natural antioxidants [12].

In the present study, we investigate antibacterial activity against *Staphylococcus aureus* and *Eschrichia coli* and study the chemical content by using GC-MS instrument of ethanol extract of *kelakai*.

MATERIALS AND METHODS

Materials

Kalakai were collected from Sungai Siring, Samarinda city, East Kalimantan. The plant was identified by a staff at the Laboratory of Physiology, Mulawarman University. All solvents were redistillated before using in this research.

Instrumentation

The GC-MS analysis Shimadzu QP-2010 Ultra gas chromatograph coupled to a mass spectrometer instrument (Shimadzu Corporation, Japan) in a system operated by electron impact (70 eV). Column Oven Temp. :60.0 °C to 290 ^{o}C, Injection Temp. :280.00 °C, Injection Mode :Split, Flow Control Mode :Linear Velocity, with helium as the carrier gas.

Extraction

Extraction methods used are similar to our previous studies. 520 gram dried powder of *Kalakai* was macerated with methanol for 3 x 24 h at room temperature. The extract was collected, filtered, and concentrated using rotary evaporator at reduced pressure to yield 31 gram ethanol extract [13,14]. Then, extracts were analyzed by GC-MS and antibacterial activity test

Antibacterial assay

Antibacterial activity of *Kalakai* extracts determined based on the value of the minimum inhibition concentration (MIC). Pure cultures of *Staphylococcus aureus* and *Escherichia coli* beforehand regenerated into a solid medium and then cultured in a liquid medium. Furthermore, cultured bacteria can be used to test the antibacterial activity. Antibacterial activity test was conducted using the agar diffusion method (Kirby-Bauer). Bacterial inoculum put in to the petri dish using a sterile swab. Bacterial inoculum smeared on the media to tilt 90 ^{o}C continuously until evenly distributed. The sterile disc (5 mm) containing the extract were placed on the agar medium. Stireile disc containing the chloramphenicol as a positive control and other without the addition of the extract as a negative control were also placed on the agar medium. Diameters of inhibition was measured after incubation at 37 ^{0}C for 24 hours [15,16].

Gas chromatography-mass spectrometry analysis

The ethanol extract was analyzed using GC-MS instrument Shimadzu QP-2010 in an operating system electron impact (70 eV). GC-MS spectrum was identified by comparing peak data base of instrument (libraries of the instrument/NIST/EPA/Mass Spectral Library).

RESULTS AND DISCUSSION

GC-MS Analysis

Gas Chromatogram spectrum of ethanol extract of *Kalakai* showed the presence of four peaks with retention time 22.005, 23.621, 24.440, 25.626 minutes. The structure of the compounds are determined based on the fragmentation pattern and compared with the library data base, the peaks 1, 2, 3, and 4 are stigmasterol (46.51%), Stigmasta-dien-5,22-3-ol, acetate, (3 beta) (14:32), Ethyl linoleate (2:57%), and Stigmast-5-en-3-ol, oleic (36.60%), respectively.

Tabel 1; Compounds identified in the ethanolic extract of *Stenochlaena palustris* by GC-MS

Peak	Retention Time (min)	Peak Area (%)	Compounds	Molecular weight	Moleculer formula
1	22.005	46.51	Stigmasterol	412	$C_{29}H_{48}O$
2	23.621	14.32	Stigmasta-5,22-dien-3-ol, acetat, (3. beta)	394	$C_{31}H_{50}O_2$
3	24.440	2.57	Ethyl linoleate	308	$C_{20}H_{36}O_2$
4	25.626	36.60	Stigmast-5-en-3-ol, oleat	679	$C_{47}H_{82}O_2$

Biological activity by compounds

Stigmasterol, unsaturated plant sterols, is a precursor in the biosynthesis pathway of many fitosteroid compounds. The previous research shows stigmasterol has a variety of interesting activities and shown antioxidant [17] and significant free radical scavenging activities [18]. It found as cytotoxicity against a brine shrimp, *A. Salina* [18,19], antibacterial activity [20], inhibit tumor promotion, anti HIV reverse transcriptase, and anti-inflammatory [21]. Stigmasterol has also can lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid [22] and inhibits several pro-inflammatory and matrix degradation mediators typically involved in OA-induced cartilage degradation [23], In previous report, it was also summarized their activity by Dandi *et al* [24]. While the stigmasterol derivative compounds (Stigmasta-5,22-dien-3-ol, acetate, (3 beta) and Stigmast-5-en-3-ol, oleic), not much reported about its activity. Only Stigmast-5-en-3-ol, oleic has a potent activator ACE [25].

Ethyl linoleate is a minor component, previously reported it can inhibit cholesterol esterification catalyzed by pancreatic cholesterol esterase and hepatic and aortic microsomal fatty acyl-CoA:cholesterol O-acyltransferase (EC 2.3.1.26) [26]. on the other hand, ethyl linoleate attenuates pro-inflammatory cytokines and induced HO-1 expression [27].

Antibacterial activity test

Zone of inhibition of antibacterial activity in the present research was shown in the table 2. Variations in the concentration of ethanolic extract of *Eschrichia* 10, 15, 20, 25, and 30 % have a diameter of inhibition of 7, 7, 8, 9, and 10 mm respectively against S. *aureus* and 7, 7, 7, 8, and 12 mm respectively against *E. coli*. MIC values for both S. *aureus* and E. coli are found to be10 % w/v.

Tabel 2. zone of inhibition of ethanol extracts of *Stenochlaena palustris* Leaves

Bacteria	zone of inhibition (mm)				
	Extracts (% w/v)				
	10 %	15 %	20 %	25 %	30 %
Staphylococcus aureus	7	7	8	9	10
Eschrichia. coli	7	7	7	8	12

CONCLUSION

Based on the results of the GC-MS spectrum analysis, ethanol extracts of *Stenochlaena palustris* leaves containing stigmasterol (46.51%), stigmasta-dien-5.22-3-ol, acetate, (3 beta) (14:32), Ethyl linoleate (2.57 %), and Stigmast-5-en-3-ol, oleic (36.60%). In some previous reports, Stigmasterol, a major compound has a variety of interesting activities. While the MIC value of ethanolic extract against both *Staphylococcus aureus* and *Eschrichia coli* same was 10 % (w/v).

REFERENCES

[1] Chambers, T.C, *Telopea Journal of Plant Systematics*, **2013**, 15, 13–36
[2] Giesen W. S., Wulffraat, M., Zierenand and Scholten, L. Mangrove Guidbook For Southeast Asia, FAO and Wetlands International, Bangkok, **2007**, 227.
[3] Rahajoe, J.S, Kohyama T**.** *Tropic*, **2003**, 12, 277-286.
[4] Miyamoto K, Suzuki E, Kohyama T, Seino T, Mirmanto E, Simbolon H. J Trop Ecol, **2003**, 19, 43-54.
[5] Lee, C.H. and S.L. Shin. Functional activities of ferns for human health. In H. Fernández, M.A. Revilla, and A. Kumar (eds.), Working with ferns: Issues and applications. Springer, New Yor, **2010**, 347-359.
[6] Irawan D., Wijaya C. H, Limin, S. H. Hashidoko, Y., Osaki, M. and Kulu, I.P. Tropics, **2006**, 15(4), 441-448.
[7] Suhartono, E., Viani, E., Ramadhan, M.A., Gultom. I. S., Rakhman, M.F., and Indrawardhana, D. *PCBEE Procedia* **2012**, 4, 235 – 239.
[8] Suhartono, E., Bakhriansyah, M., Handayani, R. *Indonesian Pharmaceutical Magazines*, **2010**, 21 (3), 166-170
[9] Z. Zuraini, S. Sasidharan, S. Roopin Kaur and M. Nithiyayini, *Pharmacology online*, **2010**, 1, 233-237
[10] Sumathy V, Jothy Lachumy S, Zuraini Z, & Sasidharan S. *Mal J Nutr*, **2010**, 16(3), 439-446.
[11] Suhartono, E., Viani, E., Rahmadhan, M.A., Gultom, I. S., Rakhman, M. M and Indrawardhana, D. *International Journal of Chemical Engineering and Applications*, **2012**, 3, (4), 297-299.
[12] Chai, T.T., Panirchellvum, E., Ong, H.C., and Wong, F.C. *Botanical Studies*, **2012**, 53: 439-446.

[13] Erwin, Noor,A., Soekamto, N.H., Altena, I.V., and Syah. Y.M. *Biochemical Systematics and Ecology*, 2014, 55, 358-361
[14] Erwin, Sulistyaningsih, S. and Kusuma, I. W., *J Pharm Bio Sci*, 2015, 6, (1), 598 – 604.
[15] Mahato, R.B. and Chaudhary, R.P. *Scientific World*, **2005**, 3 (3), 26-31
[16] Gowri, S.S. and Vasantha, K. *J. Appl. Sci. Environ. Manage*, **2009**, 13(4) 99 – 101.
[17] Shanthakumar, B., Sathish, M., and A Suresh, A. J. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2013**, 4(4), 1411-1418.
[18] Parvin, M.S., Rahman, M.S, Islam, M.S. and Rashid, M.A, *Bangladesh J Pharmacol*, **2009**, 4, 122-125.
[19] Subhadhirasakul, S. and Pechpongs, P. *Songklanakarin J. Sci. Technol*, **2005**, 27 (2), 555-561
[20] Woldeyes, S., Adane, L., Tariku, Y., Muleta, D., and Begashaw, T. *Nat Prod Chem Res*, **2012**, 1(1), 1-8
[21] Venkata Raman, B., Samuel L.A., Ardha Saradhi, M., Narashimha Rao, B., Naga Vasmi, A., Sudhakar, M., and Radhakrishnan, T.M. *Asian J Pharm Clin Res*, **2012**, 5 (2), 2012, 99-106.
[22] Batta AK, Xu G, Honda A, Miyazaki T, Salen G. *Metabolism*, **2006**, 55(3):292-9.
[23] Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C, Berenbaum F. *Osteoarthritis Cartilage*, **2010**, 18(1), 106-16
[24] Dande, P.R., Bonde, C.G. and Pandita, N. *Int J Pharm Bio Sci*, **2013**, 4(3), 1025 – 1035.
[25] Sarina P Kabade, Savinay Kumar J.C, Harish Kumar C V, SurendraBabu K.C, Abhinava J V, Guruprasad R. *International Journal of Analytical, Pharmaceutical and Biomedical Sciences*, **2014**, 3(2), 40-46.
[26] Lange, L.G. *Proc. Natl Acad. Sci. USA*, **1982**, 79, 3954-3957
[27] Park, S.Y., Seetharaman ,R., Ko, M.J., Kim, D.Y., Kim, T.H., Yoon, M.Y., Kwak, J.H., Lee, S.J., Bae, Y.S., Choi, Y.W., *International Immunopharmacology*, **2014**, Vol. 19 (2), 253-261